COLD STORAGE OF *GONATOCERUS ASHMEADI* GIRAULT: EXTENDED EMERGENCE, AND PARENTAL AND PROGENY FITNESS

Principal Investigator:
Roger A. Leopold
Biosciences Research Laboratory
USDA, ARS
Fargo, ND 58105

Cooperator:
Wen-Long Chen
Department of Entomology
North Dakota State University
Fargo, ND 58105

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ABSTRACT
The emergence pattern was changed after immature *G. ashmeadi* were stored within their *Homalodisca vitripennis* egg hosts under a fluctuating temperature and a short-day photoperiod for 30 d. The fitness of parasitoids collected from three emergence peaks coming 1-5, 12-16, and 23-26d after the onset of the first emergence was investigated by examining developmental and reproductive parameters. Likewise, these parameters were also determined for the F_1 and F_2 progeny. The development to adulthood of the parental parasitoids collected from second and third emergence peaks was delayed by approximately 106% and 279% while the parasitoids collected from first emergence period had no developmental delay. Compared to the control group, the parasitism of the egg hosts by parasitoids collected from the first, second, and third emergence periods was decreased by 43, 68 and 80%; the fecundity by 53, 84 and 89%; and the longevity by 27, 72 and 67%, respectively. The F_1 parasitoids derived from parents collected from second and third emergence periods also had a lower incidence of parasitism. The fecundity and longevity of F_1 and F_2 parasitoids derived from parents collected from second and third emergence periods were significantly reduced. However, development and emergence of the F_1, F_2 and F_3 parasitoids were not influenced by any of the periods from which their ancestors emerged.

INTRODUCTION
Dormancy is one of the major strategies employed by insects and mites to survive harsh environmental conditions (Leopold, 1998). It is an adaptive response of arthropods to adverse environmental conditions by often entering a state of diapause or quiescence. Diapause is an indirect response to unfavorable conditions. It is mediated via the endocrine system, resulting in developmental arrest and adaptive physiological changes (Blum, 1985). Development usually resumes upon exposure to the appropriate environmental signals. Quiescence is a direct response to harsh conditions and results in suppression or arrest of development. Once adverse conditions cease, the organism can quickly recover and resume immediate development (Tauber et al., 1986). Either diapause or quiescence can be used in devising cold storage methods to facilitate mass-rearing of beneficial insects in classic biological control programs.

The egg parasitoid, *Gonatocerus ashmeadi* Girault, is one of the most common natural enemies of the glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar), in California. Augmentative release of this parasitoid is a feasible approach for control of the GWSS in affected areas where other methods are not accepted or appropriate. Mass-rearing parasitoids for control of the GWSS becomes problematic when there is a shortage of host eggs because there is currently no artificial diet to rear these wasps. Shortage of GWSS eggs can occur because there is a reproductive diapause in this insect that occurs during the winter months. Oftentimes there are insufficient numbers of parasitoids in the colder areas of California to produce an impact on sharpshooter populations (Morse et al, 2005), so it is important to develop effective methods to store a large number of hosts and parasitoids to meet these fluctuating demands in the field.

The effects of cold storage on parasitized and non-parasitized eggs of the glassy-winged sharpshooter have been recently studied to aid the mass-rearing of *G. ashmeadi* (Chen and Leopold, 2007, Chen et al, 2008a). Chen et al (2008b) also developed a method to store adult *G. ashmeadi* and examined the subsequent storage effects on maternal and progeny fitness. Storage at 4.5-7.5°C for 30 d induces quiescence in parasitoids stored within *H. vitripennis* eggs deposited beneath the surface of euonymus (*Euonymus japonica* Thumb.) leaves (Chen et al, 2008a). After cold storage, emerging adults have three emergence peaks after the initial onset (Chen et al. 2008a) whereas parasitoids reared continuously at 16-32°C have only one peak (Chen et al. 2006). In this study, we examined the biological and reproductive fitness of the parental generation of this parasitoid collected separately from the three emergence peaks and also of the F_1 and F_2 progeny to determine whether the gated emergence response elicited by extended storage had an effect on the fitness of maternal and progeny *G. ashmeadi*.

OBJECTIVES
1. Determine whether the extended emergence pattern affects reproduction and/or development of the post-storage parental generation.
2. Determine whether the extended emergence pattern affects fecundity and longevity of parental, F_1 and F_2 generations and progeny development.
3. Determine the post storage incidence of parasitism, emergence pattern and sex ratio of the parents and their progeny.

RESULTS AND CONCLUSIONS

Objective 1. Adult emergence pattern and developmental time of post-storage parasitoids.

After the parasitoids were stored within their host at 4.5, 6.0 and 7.5 °C, (each temperature changing at eight h intervals over a 24 h period for 30 d) the emerging adult parasitoids displayed an emergence pattern consisting of three peaks while the control group only had one emergence peak (**Figure 1**). To determine possible effects on development after cold storage, parasitoids were collected during each period of emergence (i.e., 1-5 d; 12-16 d; and 23-26d after the initial onset of emergence). Development time in this study was measured from the removal from cold storage to the medium time of adult emergence. Wasps emerging from the control group were also collected during their emergence peak. **Figure 2** shows that development time of stored parasitoids varied significantly with the emergence periods ($F = 835.72$, $df = 3,15$, $P < 0.0001$). After storage for 30 d, more than 60% of the wasps emerged during first period and the development time was similar to the control group (**Figure 1**). These results indicate that a large of number of immature *G. ashmeadi* could quickly recover from the cold-induced quiescence and resume normal development. However, when compared to the control, the development time of wasps collected from second and third emergence periods was delayed approximately one-(106%) and three-fold (279%), respectively. Approximately 26% and 4% of parasitoids collected from the second and third emergence periods (Chen et al, 2008a) did not quickly resume development after removal from cold storage. It is uncertain what causes this significant delay in development time. These results are being studied further.

Objective 2. Parasitism, fecundity and longevity of parents and progeny.

A repeated measure ANOVA showed that the incidence of parasitism by the parasitoids varied significantly with generation ($F = 6.08$, $df = 2,56$, $P = 0.004$) and emergence period ($F = 13.73$, $df = 3,28$, $P < 0.0001$) and that there was significant interaction between generation and the emergence period of the parasitoids ($F = 2.37$, $df = 6,56$, $P = 0.042$). Parental ($F = 9.77$, $df = 3,29$, $P < 0.0001$) and the F_1 ($F = 2.78$, $df = 3,28$, $P = 0.034$) generations showed a significant decrease in parasitism of host eggs across the emergence periods. When compared to the control group, the parasitism by the wasps collected from the first, second, and third emergence periods declined by 43, 68 and 80%, respectively. There was no difference in the rate of parasitism by the F_2 generation whose grandparents were collected from different emergence periods ($F = 1.49$, $df = 3,36$, $P = 0.235$) (**Figure 3A**).

Lifetime fecundity varied significantly with generation ($F = 24.02$, $df = 2,56$, $P < 0.0001$) and the emergence period ($F = 37.55$, $df = 3,28$, $P < 0.0001$). There was a significant interaction between generation and the emergence period of parasitoids ($F = 6.07$, $df = 6,56$, $P < 0.0001$). The fecundity of parental parasitoids was significantly influenced by the period that the wasps emerged. Compared to the control group, the fecundity of parasitoids collected from the first, second, and third emergence periods decreased by 53, 84 and 89%, respectively. There was also a significant decrease in fecundity of F_1 and F_2 parasitoids derived from the parents that were collected during the second and third emergence periods (**Figure 3B**). Longevity of the parasitoid varied significantly with generation ($F = 9.81$, $df = 2,56$, $P = 0.0002$) and the emergence period ($F = 54.64$, $df = 3,28$, $P < 0.0001$). There was no significant interaction between generation and the emergence period of parasitoids ($F = 1.36$, $df = 6,56$, $P = 0.247$). The longevity of parental parasitoids was significantly influenced by the emergence period. Compared to the control group, the longevity of parasitoids collected from the first, second, and third emergence periods decreased by 27, 72 and 67%, respectively. The longevity of F_1 and F_2 parasitoids derived from the parents that were collected during the first emergence period was similar to that of the control group. However, there was a significant decrease in longevity of F_1 and F_2 parasitoids derived from the parents there collected during the second and third emergence periods (**Figure 3C**).

Objective 3. Development, emergence and sex ratio of F_1, F_2 and F_3 generations.

A repeated measures ANOVA showed that emergence of parasitoids was not significantly influenced by generation ($F = 2.45$, $df = 2,50$, $P = 0.097$) and the period ($F = 0.47$, $df = 3,25$, $P = 0.703$) that the parasitoids emerged. There was no significant interaction between generation and the emergence period of parasitoids ($F = 0.74$, $df = 6,50$, $P = 0.620$) (**Figure 4A**). The development time of parasitoids emerging from non-stored, recently collected *H. vitripennis* eggs (< 24 h old) was not significantly influenced by generation ($F = 2.81$, $df = 2,36$, $P = 0.074$) or the peak ($F = 2.37$, $df = 3,18$, $P = 0.079$) from which the parasitoids emerged.

There was no significant interaction between generation and the emergence period of parasitoids ($F = 0.74$, $df = 6,36$, $P = 0.082$) (**Figure 4B**). Data on sex ratio of F_1, F_2 and F_3 generations are not shown here because of a lack of a sufficient number of replicates. The complementary experiments are in the process of being conducted.

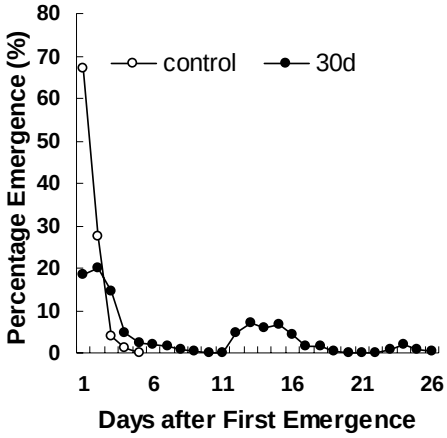


Figure 1. Adult emergence pattern of *G. ashmeadi* after being held in cold storage under the 4.5-7.5 °C daily fluctuating temperature for 30 d.

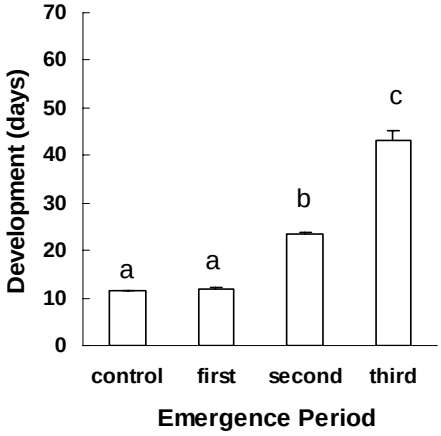
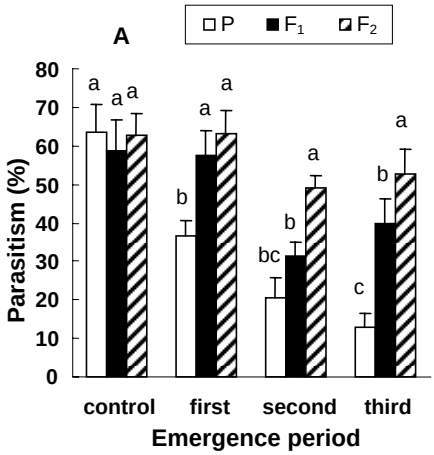
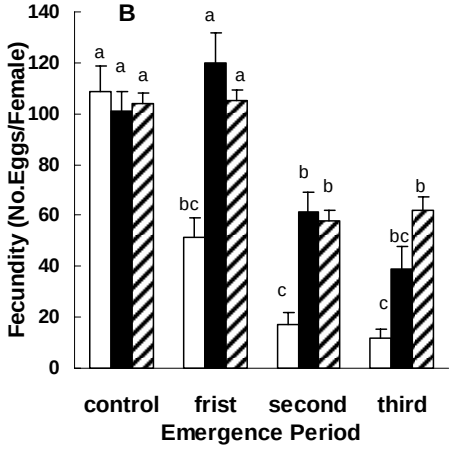
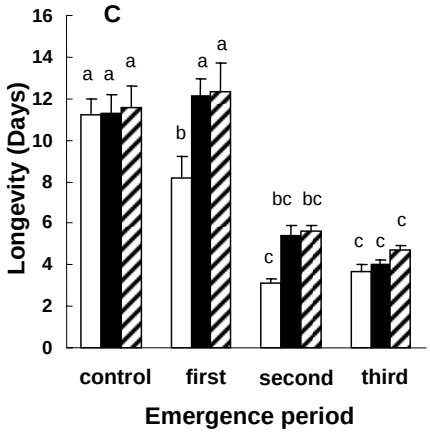


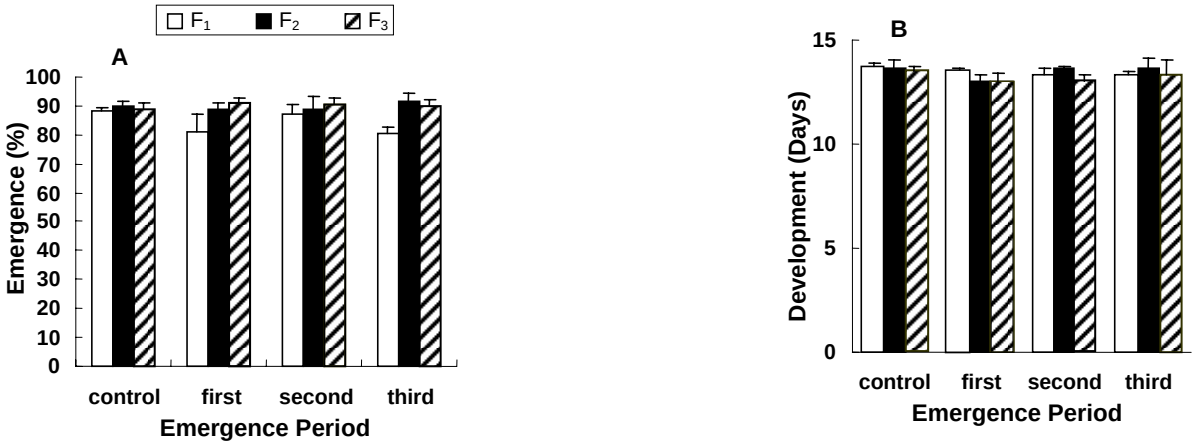
Figure 2. Development time of *G. ashmeadi* collected from various emergence periods after being held in cold storage under the 4.5-7.5 °C daily fluctuating temperature for 30 d. Columns denoted by differing letters are significantly different.







Figures 3 A-C. Parasitism (A), fecundity (B), and longevity (C) of the parental, F_1 and F_2 generations of *G. ashmeadi* collected from various emergence periods after cold storage under the 4.5-7.5 °C daily fluctuating temperature for 30 d.



Figures 4A & B. Developmental time (A), emergence (B) of the F_1, F_2 and F_3 generations of *G. ashmeadi* collected from various emergence peaks after cold storage of their ancestors that were stored under the 4.5- 7.5°C daily fluctuating temperature for 30 d.

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